Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 303 (2003) 46–51
BBRC
www.elsevier.com/locate/ybbrc

Function of the loop residue Thr792 in human DNA topoisomerase IIα☆

Namiko Suda,[a] Yoshiyuki Nakagawa,[b] Akihiko Kikuchi,[b] Masako Sawada,[c] Yuko Takami,[c] Hiro-omi Funahashi,[a] Akimasa Nakao,[a] Shonen Yoshida,[c] and Motoshi Suzuki[c,*]

[a] *The Second Department of Surgery, Nagoya University Graduate School of Medicine, Nagoya 466-8550, Japan*
[b] *Laboratory of Medical Mycology, Research Institute for Disease Mechanism and Control, Nagoya University Graduate School of Medicine, Nagoya 466-8550, Japan*
[c] *Laboratory for Cancer Cell Biology, Research Institute for Disease Mechanism and Control, Nagoya University Graduate School of Medicine, Nagoya 466-8550, Japan*

Received 10 February 2003

Abstract

We studied the mutation effect of one of the putative loop residues Thr792 in human DNA topoisomerase IIα (TOP2α). Thr792 mutants were expressed from high or low copy plasmids in a temperature sensitive yeast strain deficient in TOP2 (*top2-1*). When expressed from a high copy plasmid, mutants with small side chains complemented the yeast defect; however, from a low copy plasmid, only wild-type, Ser, and Cys substitution mutants complemented the yeast defect. Interestingly, at the permissive temperature other mutants (e.g., Val, Gly, and Glu substitutions) showed the dominant negative effect to the *top2-1* allele, which was not observed by the control α4-helix mutants. T792E mutant was 10-fold less active than wild-type and the T792P had no decatenation activity in vitro. These results suggest that Thr792 in human TOP2α is involved in enzyme catalysis.

Keywords: DNA topoisomerase; DNA replication; Chromosomal segregation; Drug resistance; Anticancer reagent; Cell division; Cell cycle

DNA molecules exist in nuclei as compact, supercoiled, and catenated structures that become relaxed or resolved during transcription and DNA replication. This topological change requires type II DNA topoisomerase, an ATP-dependent enzyme, that introduces transient incisions and rejoins double-stranded DNA to alter superhelical tension and change DNA topology [1].

In higher eukaryotic cells, TOP2 exists in two isoforms, α and β [2,3]. It is likely that TOP2α and β play roles in cell division, DNA replication, and transcription. TOP2α may play a role during chromosome condensation and partitioning, because the level of TOP2α reaches a maximum in G2/M [4]. In contrast, TOP2β is expressed at a constant level [5,6] and may play a role in cell differentiation [7,8].

Eukaryotic type II topoisomerase is a homodimer composed of subfragment A′ with a breakage/rejoining domain and subfragment B′ with an ATPase domain. The primary structure of each domain/subfragment is highly conserved among known species. Amino acid sequences and crystal structures of *Saccharomyces cerevisiae* TOP2 and *Escherichia coli* DNA gyrase share extensive similarity [9,10]. Genetic complementation tests show that mouse, *Drosophila*, and human TOP2 enzymes complement TOP2-deficient yeast cells [11–13].

The breakage/rejoining domain includes the active site Tyr805, which is a residue in the helix turn helix (HTH) winged motif [9]. In our previous work, mutants in a loop region near the active site of human TOP2α were screened for their ability to complement yeast cells with a temperature sensitive mutant of TOP2 [14]. We showed that active site Tyr805 is essential for complementation and that mutants in Leu794, Asp799, Ala801, and Arg804 complement the yeast mutant only if conservative substitutions are made. Furthermore, in the

☆ *Abbreviations:* TOP2, DNA topoisomerase II; kDNA, kinetoplast DNA.

* Corresponding author. Fax: +81-52-744-2457.
E-mail address: msuzuki@med.nagoya-u.ac.jp (M. Suzuki).

0006-291X/03/$ - see front matter © 2003 Elsevier Science (USA). All rights reserved.
doi:10.1016/S0006-291X(03)00297-3

structure model Thr792 may interact with Asp799, suggesting that Thr792 is also employed for catalysis as the other loop resides. Interestingly, the amino acids in *S. cerevisiae* TOP2 that correspond to these residues of human TOP2α lie in the same plane as active site Tyr782. (Tyr805 in human TOP2α is equivalent to yeast Tyr782.) To better understand the functions of loop residues for genetic complementation and catalysis, we genetically and biochemically analyzed the Thr792 mutations.

Materials and methods

Yeast strains, cDNA, and oligonucleotides. Complementation activity was measured in the yeast strain SD1-4 (MATa, *ade*1, *ade*2, *ura*3-52, and *top2-1*). Full-length human TOP2 cDNA was described previously [14,15]. DNA oligomers were synthesized and purified by Amersham Pharmacia Biotech or NK products (Osaka, Japan).

Plasmid construction. One of the two *Bgl*II sites in LLpYES hTOP2-PGAL1 [14] was inactivated by inserting the oligomer 5′-GATCAGTCAGTCAGTCAGTCG annealed to the complementary sequence 5′-GATCCGACTGACTGACTGACT (p′YES-PGAL1-hTOP2). A non-functional TOP2 plasmid (Dummy Vector) was constructed by ligating the large *Bgl*II–*Kpn*I fragment of p′YES-PGAL1-hTOP2 to the oligomer 5′-GATCTAGTCAGTCAGTCGAG CTCGCATGCAGTCAGTCAGTCGGTAC annealed to 5′-CGACT GACTGACTGCATGCGAGCTCGACTGACTGACTA.

For some experiments, a centromeric plasmid carrying the ADH1 promoter, YCp-PADH1-hTOP2, was constructed. The ADH1 promoter from YPB1-ADHpt was inserted into the large *Nae*I–*Not*I fragment of p′YES-PGAL-hTOP2 (p′YES-PADH1-hTOP2). The *Sac*I linker (5′-TCGAAGTCAGGAGCTCAGTCAG annealed to 5′-TCGACTGACTGAGCTCCTGACT) was inserted and p′YES-PADH1-hTOP2 was digested with *Sna*BI and *Sac*I. The large fragment was ligated with the large *Sma*I–*Sac*I fragment of YCplac33 (YCp-PADH1-hTOP2).

Site-directed mutagenesis. PCR mutagenesis was carried using PCR-A primer, 5′-AG CCC ATT GGT CAG TTT GGT NNN AGG CTA CAT G, where NNN indicates random sequence that encodes 19 different amino acids. The reverse primer is complementary to the *Xba*I site of hTOP2 cDNA (5′-C AAC ATG GGT TCT AGA ACT TG). Using LLpYES-PGAL1-hTOP2 as a template, 30 cycles of PCR were carried out (94 °C for 30 s, 54 °C for 30 s, and 72 °C for 30 s) using proofreading-competent Pyrobest DNA polymerase (TaKaRa, Kyoto, Japan). A 450-base pair PCR product was isolated by electrophoresis in 1.2% agarose and purified by QIAEXII gel extraction kit (Qiagen, Valencia, CA). A 240-base pair fragment was synthesized using the forward primer, 5′-CT GAT AAC GAG AGA TCT ATC C, which contains a *Bgl*II site, and PCR-B primer, 5′-ACC AAA CTG ACC AAT GGG CT. These two PCR fragments were used as templates to amplify the 680-base pair fragment using forward and reverse primers. The fragment was digested by *Bgl*II and *Xba*I and ligated with the large *Bgl*II–*Xba*I fragment of the Dummy Vector. Constructs were isolated and sequenced. The double mutant T792D:D799T was constructed using primer 5′-AG CCC ATT GGT CAG TTT GGT GATAGG CTA CAT GGT GGC AAG ACCTCT GCT AGT CCA for PCR-A primer; underlined nucleotides introduce amino acid substitutions.

Mutations were transferred into a centromeric plasmid as follows: another dummy vector, YCp-PADH1-dum, was constructed by ligating the larger *Not*I–*Kpn*I fragment from YCp-PADH1-hTOP2 with 5′-GGCCGCAGTCAGGGGCCCAGTCAGACGCGTAGTCAGG TAC annealed to 5′-CTGACTACGCGTCTGACTGGGCCCCTGA CTGC. The smaller *Not*I–*Pac*I fragment of YCp-PADH1-dum was replaced with the *Not*I–*Pac*I fragment from each p′YES-PGAL-hTOP2 mutant.

The α4 helix motif mutants were also constructed by site-directed mutagenesis. In this study, mutants that did not complement the SD1-4 strain were chosen and used for analysis. Detailed construction method will be reported elsewhere.

Genetic complementation of top2-deficient yeast. The complementation assay was described previously [14]. Plates were cultured at 35 °C for 3 days and colonies were counted. Three independent experiments were performed with independent clones.

Cell growth. SD1-4 cells were transformed with a centromeric plasmid expressing mutant TOP2α. Fresh transformants were inoculated into SCglu (6.7 g/liter yeast nitrogen base without amino acids, 5 g/liter casamino acids, 20 g/liter glucose, 20 mg/liter adenine sulfate, 20 mg/liter tryptophan, and 20 mg/liter uracil) and cultured overnight at 25 °C. Cells were washed with water, diluted to OD_{600} at 0.05 in SCglu with or without uracil, and cultured at 25 °C. The rate of cell growth was determined from turbidity measurements taken at the indicated time points.

Plating efficiency. SD1-4 cells were transformed with a centromeric plasmid expressing mutant TOP2α. Fresh transformants were inoculated into SCglu and cultured overnight at 25 °C. Cell aliquots were diluted, plated onto SCglu with or without uracil plates, and cultured at 25 or 35 °C for 3 days.

Enzyme purification. Expression and purification of wild-type and mutant TOP2 was carried out as described previously with modifications [15] using BAC-TO-BAC HT Baculovirus Expression System (Life Technologies, MD). At 72 h postinfection, 2×10^8 cells were harvested by centrifugation at 600*g*, washed with 30 ml phosphate-buffered saline [PBS; 137 mM NaCl, 2.68 mM KCl, 8.1 mM Na_2HPO_4, and 1.47 mM KH_2PO_4)], lysed in 18 ml ice cold-lysis buffer [50 mM KCl, 10 mM Tris–HCl (pH 7.5), 2 mM $MgCl_2$, 1% Triton X-100, 10 mM of 2-mercaptoethanol, and 0.15 mg/ml phenylmethylsulfonyl fluoride (PMSF)] at 4 °C, and homogenized (40 strokes) on ice in a tight fitting Dounce homogenizer. The sample was centrifuged at 600*g* for 10 min. The pellet (nuclei) was resuspended in 12 ml resuspension buffer [50 mM KCl, 10 mM Tris–HCl (pH 7.5), 2 mM $MgCl_2$, 10 mM of 2-mercaptoethanol, and 0.15 mg/ml PMSF] and centrifuged at 600*g* for 10 min. The pellet was washed and resuspended in 6 ml resuspension buffer-2 [50 mM KCl, 10 mM Tris–HCl (pH 7.5), 2 mM $MgCl_2$, 16 mM of 2-mercaptoethanol, 0.4 mg/ml PMSF, 5 mM EGTA]. Six ml of 2× nuclear extraction buffer [2 M NaCl, 80 mM Tris–HCl (pH 7.5), and 20% glycerol] and 6 ml PEG buffer [18% PEG 8000, 1 M NaCl, and 10% glycerol] were added slowly with stirring in this order. The mixture was stirred for 30 min and centrifuged at 10,000*g* for 10 min. The supernatant was loaded onto a 2 ml In-resin column (Nonagon, His-Bind Resin, WI) equilibrated with buffer A [20 mM Tris–HCl (pH 8.5), 500 mM KCl, 20 mM imidazole, 10 mM of 2-mercaptoethanol, and 10% glycerol]. The column was washed with 20 ml buffer A, 0.6 ml buffer B [20 mM Tris–HCl (pH 8.5), 1 M KCl, 10 mM 2-mercaptoethanol, and 10% glycerol] and 6 ml buffer A. Bound protein was eluted with elution buffer [20 mM Tris–HCl (pH 8.5), 100 mM KCl, 1 M imidazole, 10 mM of 2-mercaptoethanol, and 10% glycerol] and 500 μl fractions were collected. Peak fractions were dialyzed against 500 ml buffer-D1 [25% glycerol, 50 mM potassium phosphate (pH 7.7), 0.5 mM DTT, 1 mM EDTA, and 100 mM NaCl] for 2 h and against buffer-D2 [50% glycerol, 50 mM potassium phosphate (pH 7.7), 0.5 mM dithiothreitol (DTT), 1 mM EDTA, 1 mM EGTA, and 100 mM NaCl] for 2 h. Protein concentration was determined with Bradford reagent (BioRad, Richmond, CA) and the enzyme was stored at −20 °C for months.

Topoisomerase activity assay. Decatenation activity was measured using kinetoplast DNA (kDNA) (TopoGEN, Columbus, OH). A 20 μl decatenation reaction contained 100 ng kDNA in 50 mM Tris–HCl (pH 8.0), 120 mM KCl, 10 mM $MgCl_2$, 0.5 mM DTT, 30 μg/ml BSA, and 1 mM ATP. Enzyme was added and incubated for 15 min at 37 °C.

The reaction was stopped with 2 μl loading dye (1% SDS, 50% glycerol, and 0.05% bromophenol blue, TaKaRa). Reaction products were separated by electrophoresis in 1% agarose gel in the presence of 1.0 μg/ml ethidium bromide. Agarose gels were destained in water for 45 min and reaction products were visualized under UV light. One unit of decatenation activity is defined as the amount of enzyme that decatenates 100 ng kDNA under these conditions.

Results and discussion

Genetic complementation of top2-deficient yeast by Thr792 mutants of human TOP2

Human TOP2α Thr792 and Asp799 are conserved residues positioned in a putative loop region in the helix turn helix (HTH) winged motif. In *S. cerevisiae* TOP2, the analogous residues lie in close proximity (Figs. 1 and 2), but are distant from the catalytic tyrosine. Previous studies show that Asp799 is essential for catalysis by human TOP2α [14]. To identify the role of Thr792 and functions of human TOP2α, we introduced mutations in this enzyme.

Plasmids expressing mutant or wild-type human TOP2α were constructed in a high copy vector pYES2 and transformed into temperature-sensitive *top2-1* yeast. At the non-permissive temperature, complementation was observed for wild-type TOP2α and a control mutant D799E [14]. A double mutant of TOP2α T792D:D799T was also constructed, in which the identity of the two residues is switched. In this mutant, a hydrogen bond may form between the two mutant residues Asp792 and Thr799. The T792D:D799T mutant of human TOP2α did not complement *top2-1* yeast at the restrictive temperature (Fig. 3A).

	▽ 792	793	▼ 794	795	796	797	▼ 798	799	▼ 800	801	802	▼ 803	▼ 804	* ▼ 805	806	807	808
human TOP2α	792 T	R	L	H	G	G	K	D	S	A	S	P	R	Y	I	F	T
CG TOP2α	791 T	R	L	H	G	G	K	D	S	A	S	P	R	Y	I	F	T
Mm TOP2α	791 T	R	L	H	G	G	K	D	S	A	S	P	R	Y	I	F	T
Rn TOP2α	790 T	R	L	H	G	G	K	D	S	A	S	P	R	Y	I	F	T
human TOP2β	808 T	R	L	H	G	G	K	D	A	A	S	P	R	Y	I	F	T
Cl TOP2β	801 T	R	L	H	G	G	K	D	A	A	S	P	R	Y	I	F	T
Mm TOP2β	801 T	R	L	H	G	G	K	D	A	A	S	P	R	Y	I	F	T
Dm TOP2	772 T	R	L	S	G	G	K	D	C	A	S	A	R	Y	I	F	T
Ce TOP2β	827 T	R	L	Q	G	G	K	D	S	A	S	A	R	Y	I	F	T
Ce TOP2	829 T	R	H	E	G	G	N	D	A	A	S	A	R	Y	I	F	T
At TOP2	781 T	R	T	S	G	G	K	D	S	A	S	A	R	Y	I	F	T
Sc TOP2	769 T	R	A	T	G	G	K	D	A	A	A	A	R	Y	I	N	T
Tb TOP2	758 S	R	Q	Q	L	G	N	D	H	A	A	P	R	Y	I	F	T
TC TOP2	758 S	R	Q	Q	L	G	N	D	H	A	A	A	R	Y	I	F	T
Cf TOP2	762 S	R	Q	Q	L	G	N	D	H	A	A	P	R	Y	I	F	T

Fig. 1. Amino acid alignment of the loop region in TOP2. Protein sequences of topoisomerase II from the indicated species were aligned [21]. Amino acid coordinates in human TOP2α are indicated from Thr792 to Thr808, which corresponds to residues 769–785 in *S. cerevisiae* Top2. Numbers of the first amino acids of other TOP2 species are also indicated. Species abbreviations are as follows: *CG*, *Cricetulus griseus*; *Mm*, *Mus musculus*; *Rn*, *Rattus norvegicus*; *Cl*, *Cricetulus longicaudatus*; *Dm*, *Drosophila melanogaster*; *Ce*, *Caenorhabditis elegans*; *At*, *Arabidopsis thaliana*; *Sc*, *S. cerevisiae*; *Tb*, *Trypanosoma brucei*; *Tc*, *Trypanosoma cruzi*; and *Cf*, *Crithidia fasciculata*. Asterisk indicates the active site Tyr. The open triangle indicates Thr792 in human TOP2α. Closed triangles show catalytically important amino acids [14].

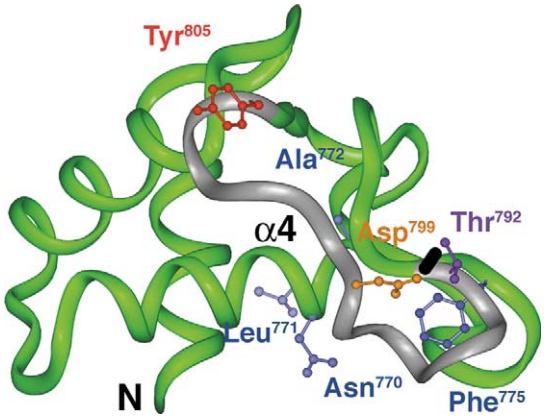

Fig. 2. Structural model based on *S. cerevisiae* TOP2. The winged motif of the A′ subfragment in the *S. cerevisiae* crystal structure (AA702–AA792) is shown. Side chains and numbers are from human TOP2α: Tyr805 (red), Asn770, Leu771, and Ala772 (blue). Thr792 and Asp799 are shown in purple and dark yellow, respectively, and the putative interaction between them is indicated by a solid bar. The polypeptide backbone between Thr792 and Tyr805 is highlighted in gray, which is referred to as 'loop region' in the text, and the remaining region is in green. N and α4 indicate the protein N-terminus and the fourth helix of the winged motif, respectively.

Human TOP2α mutants of Thr792 were constructed by site-directed mutagenesis (Thr792 mutants) and the mutants were tested in yeast complementation assays. Mutants in which Thr was substituted by Ala, Val, Gly, Ser, Cys, Glu or Asn complemented the yeast defect efficiently at 35 °C, but other amino acid substitutions were less efficient (Fig. 3A). When expressed from a low copy plasmid YCp, Thr792 mutants with Ala, Cys or Ser substitutions grew as efficiently as wild-type. In contrast, Thr792 mutants with Glu, Val, or Gly substitutions inhibited yeast growth (Figs. 3B and C). These mutants were also associated with variety of sizes and shapes when they were observed using microscope (data not shown). Therefore a small side chain at position 792 is required for complementation of *top2-1* yeast.[1]

Interestingly, the growth suppression was observed not only at the restrictive temperature, but also at the permissive temperature. Therefore mutation effect at this position was dominantly negative to the yeast cell growth. The strongest effect was observed in the yeast with T792P mutant; after the transformation, no yeast cell grew at the permissive temperature and no transformant was obtained for the analysis described below (data not shown). This effect was considered to be *top2-1* allele specific, because the effect was not observed when the mutants were introduced in yeast strains with wild-type TOP2 allele (data not shown). To know if this phenotype is common to the human TOP2α mutants in

[1] Side chains was classified according to Molecular Cloning [20].

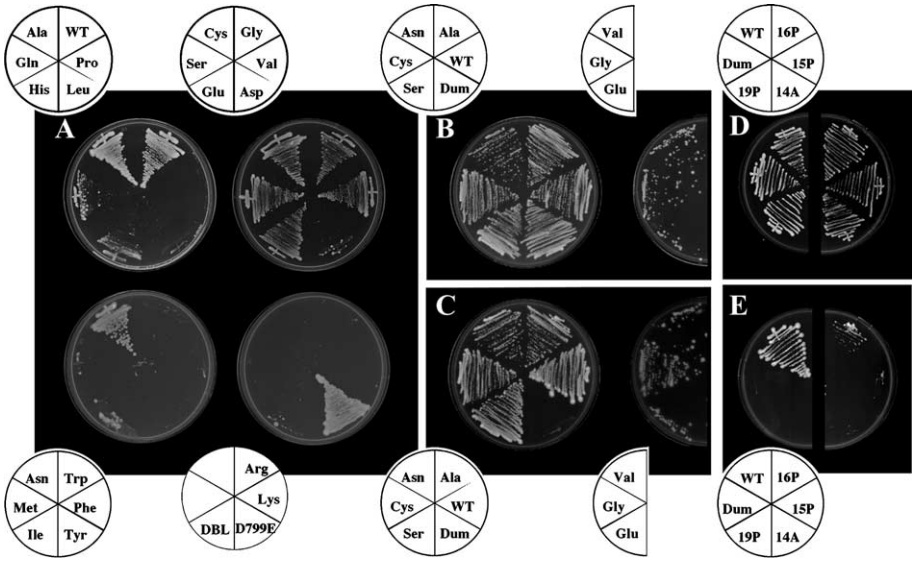

Fig. 3. Functional complementation of *top2-1* yeast by human TOP2α. (A) *S. cerevisiae* SD1-4 (*top2-1*) was transformed with WT or mutant human TOP2α with substitutions at position Thr792 expressed from high copy plasmid. Transformed cells were streaked on SCgal-URA [14] and incubated at 35 °C. The amino acid change at Thr792 is indicated at the upper or lower left of the plates. DBL indicates the double mutant T792D:D799T. (B–E) Complementation assay was performed using the low copy number YCp vector at 25 (B and D) or 35 °C (C and E). The amino acid change at Thr792 is indicated at the upper left of the plates. Dum, 14A, 15P, 16P, and 19P represent the dummy vector YCp-PADH1-dum (Materials and methods), N770A, L771P, A772P, and F775P, respectively. In (D) and (E), mutation was introduced in or near the putative α4 helix.

the *top2-1* strain, we further performed the complementation assay using other mutants, which have single amino acid substitutions in or near the putative fourth helix (α4-helix mutants, Figs. 3D and E). These mutants did not support the yeast growth at the restrictive temperature, but in contrast to the Thr792 mutants, they did not give any apparent effect on the cell growth at the permissive temperature.

Function of polar side chain at Thr792

The growth rate of Thr792 and α4 helix mutants expressed from the centromeric YCp plasmid was determined in liquid culture. Thr792 mutants with Ala, Cys, and Ser substitutions grew at a similar rate as wild-type, mutants with Asn and Gly substitutions grew at a 3- to 10-fold slower rate than wild-type, and no growth was observed when *top2-1* yeast were complemented by other Thr792 mutants with Glu and Val substitutions (Fig. 4A). Again, the α4-helix mutants grew as efficiently as wild-type at the permissive temperature (Fig. 4B).

The plating efficiency of *top2-1* yeast complemented by Thr792 mutants was determined by culturing cells in liquid medium containing uracil and plating the cells in the absence of uracil. Plating efficiency was similar in cells expressing wild-type, Cys, or Ser mutants of human TOP2α. Cells expressing the Thr792 mutant with an Ala substitution had a ~30% plating efficiency and other Thr792 mutants (Asn, Val, Gly, Gln, and Glu) did not form colonies at all (Fig. 4C). This result suggests that an uncharged polar residue (Thr, Cys, or Ser) is required at position 792, either to prevent plasmid loss or to suppress the dominant negative effect to the host chromosome replication/segregation.

It was shown that plating efficiency of the Thr792 mutants was temperature-independent. In contrast that of the α4 helix mutants and dummy vector (YCp-PADHI-dum) was dependent on temperature; i.e., at the permissive temperature it was as high as wild-type and at the non-permissive temperature, colony formation was not observed (Fig. 4C).

Decatenation activity of wild-type and mutant human TOP2α

The ability of an enzyme to complement a genetic deficiency usually reflects the ability of the protein to perform the catalysis (see [16–18]). However, TOP2 is a component of the mitotic nuclear scaffold [19], thus it may play a structural role rather than performing an enzymatic function. In order to know if enzyme activity of Thr792 mutants is required for the genetic complementation, the enzymatic capacity of T792E and T792P was analyzed using purified recombinant protein and a decatenation assay (Figs. 5A and B). Using wild-type TOP2α, maximum decatenation activity was observed with 1 ng protein. In contrast, T792E, which complemented *top2*-deficient yeast when expressed from a high copy number but not from a low copy number plasmid, showed activity 10-fold lower than wild-type. T792P did not complement *top2*-deficient yeast and had no decatenation activity at high enzyme concentration (250 ng).

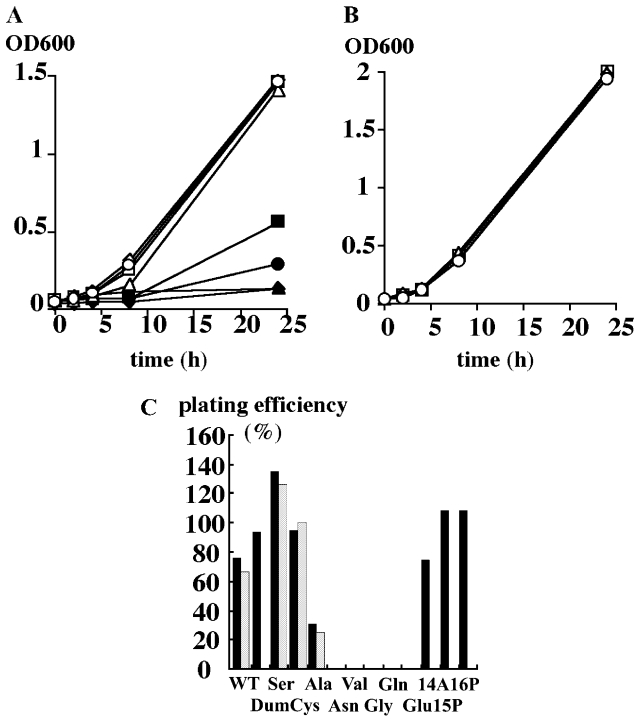

Fig. 4. Rate of growth in culture and plating efficiency of wild-type and mutant human TOP2. An overnight culture of SD1-4 expressing mutant TOP2α was inoculated in SCglu without uracil supplement and cultured at 25 °C. Cell growth was monitored at each time point. (A) Open circle, open rectangle, open square, open triangle, closed circle, closed rectangle, closed square, and closed triangle represent WT, T792S, T792C, T792A, T792G, T792V, T792N, and T792E, respectively. (B) Open circle, open rectangle, open square, and open triangle represent WT, N770A, L771P, and A772P, respectively. (C) Plating efficiency. An overnight culture of SD1-4 expressing mutant TOP2α was diluted and plated on SCglu-URA agar and cultured at 25 (solid bar) or 35 °C (open bar). Plating efficiency was determined by normalizing the number of colonies to the number of colonies grown on SCglu agar (uracil supplemented) at 25 °C (100%). Dum, 14A, 15P, and 16P represent the dummy vector YCp-PADH1-dum, N770A, L771P, and A772P, respectively. The amino acid substitution at position 792 of human TOP2α is indicated on the abscissa as codon names.

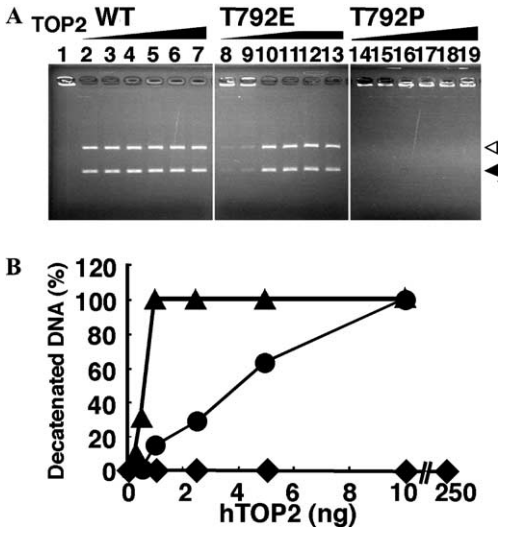

Fig. 5. In vitro activity of purified TOP2α. (A) Decatenation assay using wild-type (lanes 2–7), T792E (lanes 8–13), T792P (lanes 14–19), or no protein (lane 1). kDNA (100 ng) was incubated with TOP2 protein (lanes 2, 8, and 14; 1 ng: lanes 3, 9, and 15; 2.5 ng: lanes 4, 10, and 16; 5 ng: lanes 5, 11, and 17; 10 ng: lanes 6, 12, and 18; 50 ng: and lanes 7, 13, and 19; 100 ng) for 15 min at 37 °C. Decatenated product co-migrates with open circular (open arrow head) and covalently closed circular (closed arrow head) minicircle DNA. The DNA substrate (catenated kDNA) does not enter the gel. (B) Reaction product was quantified by densitometry and Lane Analyzer (ATTO, Tokyo, Japan). The relative amount of reaction product (vertical axis) was plotted against the quantity of enzyme (horizontal axis). Closed triangle, circle, and rectangle represent wild-type, T792E, and T792P, respectively.

These results demonstrate that Thr792 is required for catalysis by human TOP2α and suggest that the enzymatic activity of Thr792 mutants is required for complementation of *top2-1* yeast.

In this manuscript, we showed that for cell growth the plasmid-borne Thr792 mutant allele is dominant to the yeast chromosomal *top2-1* allele (Figs. 3B, C, and 4). The dominant negative phenotype would appear if an inactive mutant protein binds and occupies the TOP2α binding sites more strongly than wild-type. In the presence of a 250-fold excess of inactive T792P, activity of wild-type TOP2α was not inhibited in vitro (data not shown). Therefore, it is not likely that the mutation directly changed the affinity to the DNA substrate, although we hypothesized that the loop residues may interact with the DNA substrate [14]. Alternatively, the mutant TOP2α (human) might form a chimeric dimer with yeast TOP2, and thus, cause a dominant-negative phenotype. This is also unlikely, because not all the TOP2α gave the same phenotype; the control α4-helix mutants did not suppress the yeast cell growth at the permissive temperature. We prefer the thinking that the Thr792 mutation inhibits the interaction between TOP2α and other essential yeast protein(s), whether directly or indirectly. However, at present, other mechanisms are equally possible, e.g., TOP2α stability could differ between the mutants, depending on where and what kind of amino acid substitution takes place. Further experiments must be carried out to elucidate what mechanism underlies the dominant and non-dominant ways of complementation.

Acknowledgments

We thank Ms. Tazuko Tomita for sequencing support, Tatsuhiro Hirano for technical assistance, and Yasutomo Ito for constructing a model structure. This work was supported by Aichi Cancer Research Foundations.

References

[1] J.C. Wang, DNA topoisomerases, Annu. Rev. Biochem. 54 (1985) 665–697.
[2] M. Tsai-Pflugfelder, L.F. Liu, A.A. Liu, K.M. Tewey, J. Whang-Peng, T. Knutsen, K. Huebner, C.M. Croce, J.C. Wang, Cloning

and sequencing of cDNA encoding human DNA topoisomerase II and localization of the gene to chromosome region 17q21-22, Proc. Natl. Acad. Sci. USA 85 (1988) 7177–7181.

[3] J.R. Jenkins, P. Ayton, T. Jones, S.L. Davies, D.L. Simmons, A.L. Harris, D. Sheer, I.D. Hickson, Isolation of cDNA clones encoding the beta isozyme of human DNA topoisomerase II and localisation of the gene to chromosome 3p24, Nucleic Acids Res. 20 (1992) 5587–5592.

[4] N. Yabuki, H. Sasano, K. Kato, S. Ohara, T. Toyota, H. Nagura, M. Miyaike, N. Nozaki, A. Kikuchi, Immunohistochemical study of DNA topoisomerase II in human gastric disorders, Am. J. Pathol. 149 (1996) 997–1007.

[5] K. Kimura, M. Saijo, M. Ui, T. Enomoto, Growth state- and cell cycle-dependent fluctuation in the expression of two forms of DNA topoisomerase II and possible specific modification of the higher molecular weight form in the M phase, J. Biol. Chem. 269 (1994) 1173–1176.

[6] K. Tsutsui, K. Tsutsui, S. Okada, M. Watanabe, T. Shohmori, S. Seki, Y. Inoue, Molecular cloning of partial cDNAs for rat DNA topoisomerase II isoforms and their differential expression in brain development, J. Biol. Chem. 268 (1993) 19076–19083.

[7] X. Yang, W. Li, E.D. Prescott, S.J. Burden, J.C. Wang, DNA topoisomerase IIβ and neural development, Science 287 (2000) 131–134.

[8] K. Tsutsui, K. Sano, A. Kikuchi, A. Tokunaga, Involvement of DNA topoisomerase IIβ in neuronal differentiation, J. Biol. Chem. 276 (2001) 5769–5778.

[9] J.M. Berger, S.J. Gamblin, S.C. Harrison, J.C. Wang, Structure and mechanism of DNA topoisomerase II, Nature 379 (1996) 225–232 [published erratum appears in Nature 1996 Mar 14;380(6570):179].

[10] J.H. Morais Cabral, A.P. Jackson, C.V. Smith, N. Shikotra, A. Maxwell, R.C. Liddington, Crystal structure of the breakage-reunion domain of DNA gyrase, Nature 388 (1997) 903–906.

[11] N. Adachi, M. Miyaike, H. Ikeda, A. Kikuchi, Characterization of cDNA encoding the mouse DNA topoisomerase II that can complement the budding yeast top2 mutation, Nucleic Acids Res. 20 (1992) 5297–5303.

[12] E. Wyckoff, T.S. Hsieh, Functional expression of a *Drosophila* gene in yeast: genetic complementation of DNA topoisomerase II, Proc. Natl. Acad. Sci. USA 85 (1988) 6272–6276.

[13] Y. Hsiung, M. Jannatipour, A. Rose, J. McMahon, D. Duncan, J.L. Nitiss, Functional expression of human topoisomerase IIα in yeast: mutations at amino acids 450 or 803 of topoisomerase IIα result in enzymes that can confer resistance to anti-topoisomerase II agents, Cancer Res. 56 (1996) 91–99.

[14] Y. Okada, Y. Ito, A. Kikuchi, Y. Nimura, S. Yoshida, M. Suzuki, Assignment of functional amino acids around the active site of human DNA topoisomerase IIα, J. Biol. Chem. 275 (2000) 24630–24638.

[15] Y. Okada, A. Tosaka, Y. Nimura, A. Kikuchi, S. Yoshida, M. Suzuki, Atypical multidrug resistance may be associated with catalytically active mutants of human DNA topoisomerase IIα, Gene 272 (2001) 141–148.

[16] B. Kim, T.R. Hathaway, L.A. Loeb, Human immunodeficiency virus reverse transcriptase. Functional mutants obtained by random mutagenesis coupled with genetic selection in *Escherichia coli*, J. Biol. Chem. 271 (1996) 4872–4878.

[17] M. Suzuki, A.K. Avicola, L. Hood, L.A. Loeb, Low fidelity mutants in the O-helix of *Thermus aquaticus* DNA polymerase I, J. Biol. Chem. 272 (1997) 11228–11235.

[18] M. Suzuki, S. Yoshida, E.T. Adman, A. Blank, L.A. Loeb, *Thermus aquaticus* DNA polymerase I mutants with altered fidelity. Interacting mutations in the O-helix, J. Biol. Chem. 275 (2000) 32728–32735.

[19] W.C. Earnshaw, B. Halligan, C.A. Cooke, M.M. Heck, L.F. Liu, Topoisomerase II is a structural component of mitotic chromosome scaffolds, J. Cell Biol. 100 (1985) 1706–1715.

[20] J. Sambrook, E.F. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, second ed., Cold Spring Harbor Laboratory Press, New York, 1989, D.6.

[21] P.R. Caron, Appendix: Compendium of DNA Topoisomerase Sequence, in: M.-A. Bjornsti, N. Osheroff (Eds.), Protocols in DNA Topology and Topoisomerases, Part I: DNA Topology and Enzymes, Methods in Molecular Biology, Humana Press, Totowa, NJ, 1999, pp. 279–316.